ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

A CROSS-SECTIONAL STUDY OF BOVINE MASTITIS IN AND AROUND BAHIR DAR AND ANTIBIOTIC RESISTANCE PATTERNS OF MAJOR PATHOGENS

BY
GIZAT ALMAW ENYEW

JUNE, 2004
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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in the partial fulfillment for the requirements for the Degree of Master of Science in Tropical Veterinary Medicine

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NMC: National Mastitis Council

IDF: International Dairy Federation

BoA: Bureau of Agriculture of Region Three

CSA: Central Statistical Authority

SCC: Somatic Cell Count

ILRI: International Dairy Federation

LS: Linear Score

E.C. Ethiopian Calander

ABSTRACT

Three hundred fifty one (195 local zebu and 156 Holstein x Local Zebu) lactating cows of smallholder private farms in Bahir Dar milk shed were examined from September, 2003 to March, 2004 to determine mastitis prevalence, isolate pathogens involved, evaluate the antibiotic susceptibility profiles and to evaluate somatic cell count in identifying intramammary infections. Clinical prevalence was determined through examination of abnormalities of milk, udder or cow. California mastitis test (CMT) and culture were used for subclinical mastitis determination. Agar disc diffusion was used for antibiotic susceptibility test. Somatic cell count was conducted following standard procedures described in International Dairy Federation for enumeration of cells with direct Microscopic method.

Clinical prevalence at cow level was 3.9% in crossbreds and none in local zebu breeds. Subclinical mastitis at cow level based on CMT was high (34.4%) in crossbreds compared to indigenous zebu (17.9%) ($p < 0.05$). Quarter subclinical prevalence based on CMT was 17.90% and 4.95% for crossbreds and local zebu, respectively. Among potential risk factors considered, stage of lactation, parity and breed were found to affect the occurrence of mastitis significantly ($p < 0.05$). The pathogens isolated from mastitic milk were coagulase negative staphylococci (CNS), *S. aureus, Str. agalactiae, Str. dysgalactiae, Str. uberis,* Micrococcus species, *C. bovis, A. pyogens, B. cereus,* and *S. intermedius.* Among these, the most frequent isolates were CNS (49.6% of the total isolates), *S. aureus* (17.9%), *Str. agalactiae* (8.2%) and *Str. dysgalactiae* (6.7%).

Seven antibiotics including sulfisoxazole, tetracycline, erythromycin, oxacillin, chloramphenicol, clindamycin, and streptomycin were tested on 81 isolates. Except for streptomycin, all isolates were sensitive to all antibiotics. All isolates were most sensitive to sulfisoxazole. *Staphylococcus aureus* was susceptible to all drugs except streptomycin. S*taphylococcus aureus* was 91.7% susceptible to oxacillin, however, CNS were less susceptible (68.2%) *in vitro*.

To evaluate somatic cell count (SCC) in identifying intramamary infection for crossbreds, 10 cutoff points between 100000 cells/ ml and 300000 cells/ml were evaluated for their sensitivity and specificity in comparison with cultural results. Similarly, cutoff points between 80000 cell/ml and 250000 cell/ml were taken for local zebu breeds. The sensitivity for

crossbreds range from 79.16 % (at 300000 cells/ml cutoff point) to 95.80 % (at 100000 cells/ml) and similarly specificity from 80.28% to 45.52%. For local zebu breeds in the same order, sensitivity range from 58.82% to 88.23% and specificity from 45.82% to 85.15%. To establish threshold level this study was a first attempt in Ethiopia and to use SCC as a diagnostic tool on a national scale further study need to be conducted with improved cultural technique and automatic cell counters. Cross breed cows had high SCC compared to local zebu cows. The SCC was significantly ($p<0.05$) affected by infection status, breed and late lactation stage.

Keywords: clinical / subclinical mastitis/ prevalence/ bacterial isolate/ antibiotic susceptibility / Somatic Cell Count / threshold / crossbred / zebu

1. INTRODUCTION

Despite many years of research, mastitis remains the most economically damaging disease for dairy industry worldwide (Owens *et al.,* 1997). Based on the surveillance carried out in England and Wales from 1979 to 1982, the average cost of a case of mastitis due to antibiotics used, milk discarded, reduction in quality and the reduced milking potential of the cow for the remainder of lactation, was estimated at 60 pound for each case (Blowey, 1990). In United States, economic losses from mastitis have been calculated at approximately 200 dollar per cow per year or 2 billion per year for the nation (Seykora and McDaniel, 1985). Mungube (2001) estimated the economic losses from mastitis in the urban and periurban areas of Addis Ababa, Ethiopia, to be 210.8 birr per cow per lactation.

In addition to its economic impact, there is a danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or in rare cases provide a mechanism of spread of disease to humans. Tuberculosis and streptococcal sore throat may be spread in this way (Radostits *et al.,* 1994a). Toxic shock syndrome toxin (TSST) produced by S*taphylococcus aureus* was detected by Takeuchi *et al*. (1998) in 25 (51.8 %) of 43 isolates from clinical mastitic cows milk and in 79 (79.7%) of 126 isolates from farm bulk milk. Stephan *et al.* (2001) also obtained 34 strains of enterotoxin producing *S. aureus* obtained from milk samples of dairy cows suffering from mastitis in northeast Switzerland. Antibiotic residues following treatment of mastitis can be a potential hazard to humans in allergic reaction and possible transfer of resistance to other organisms (Hagstad and Hubbert, 1986). Due to these profound economic and public health consequences, mastitis is still a concern.

Despite Ethiopia is the most populous country in cattle than any African country, up to 1997 the per capita milk consumption was 16 kg which was lower than other countries in the region (Asfaw, 1997). In the livestock development policy to improve the per capita milk consumption, improvement of the genetic potential of the indigenous zebu through breeding with high-grade exotics was included (Asfaw, 1997). This clearly indicates that in the years to come a significant percentage of dairy cattle population in Ethiopia will be improved breeds, which are susceptible to most diseases including mastitis. Intensive market oriented periurban dairy production, a rapidly growing system in many African countries, is subject to the emergence of diseases of intensification (ILRI, 1995).

In Ethiopia, the available information indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows. According to Lemma *et al*. (2001) of the major diseases of crossbred cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases, in which 171 cows out of 556 were found to be affected. Generally, the prevalence of clinical and subclinical mastitis in different parts of Ethiopia range from 1.2 to 21.5% and 19 to 46.6%, respectively (Hussein *et al.,* 1997; Bishi, 1998; Kassa *et al.,* 1999; Lemma *et al.,* 2001; Mungube, 2001; Workineh *et al*., 2002; Kerro and Tareke, 2003). In Bahir Dar, Mekuria (1986) reported 4.9% clinical and 45.5% subclinical mastitis. In the same study area after ten years a prevalence of 40% subclinical mastitis was reported by Shirmeka (1996).

These limited studies showed that bovine mastitis is among the problems hindering dairy productivity in Ethiopia and this requires the development of methodologies of control program under the prevailing husbandry system. However, according to Hussein *et al.* (1997) so far efforts have been concentrated only on the treatment of clinical cases. On the other hand, losses from mastitis have been attributed mainly to decreased milk production from subclinical mastitis (Degraves and Fetrow, 1993).

Monitoring udder health is an important component of mastitis control (Radostitis *et al.,* 1994b). A regular assessment of udder health status is available through the use of somatic cell count (SCC) data. By setting goals for udder health status, it is easy to measure the success of udder health management programs or interventions. The practical use of SCC data to determine cow infection status requires the selection of a threshold level, which used to classify infected and healthy quarters or cows (Dohoo and Meek, 1982). Emanuelson (1997) used a threshold of 200,000 cell/ml at cow level in monitoring udder health status in Sweden. According to Dohoo and Meek (1982) 300,000 cells/ml and 250,000 cells/ml can be used to identify infected quarters and cows respectively. To the author knowledge, there are no published reports that have established this figure in local and crossbred cows under the prevailing husbandry and environmental condition in Ethiopia.

Because of the diverse bacterial etiologies of the disease, a variety of control methods involving hygiene prior to, during and after milking are used to minimize exposure of cows to mastitis causing organisms. Despite these procedures, new cases of mastitis invariably occur and antimicrobial therapy plays a role in the control of bovine mastitis. Therapy decisions are usually made empirically and often based on previous susceptibility information for the herd

in question. Rarely does the veterinarian have the chance of microbial identification and susceptibility reports to guide initial therapy decisions. This susceptibility information on susceptibility trends for bacterial species within a given herd is important (Owens *et al.,* 1997). In addition to somatic cell count, information regarding the prevalence of mastitis pathogens and antibacterial resistance or their relative frequencies over time in and around Bahir Dar was scant except the unpublished reports on prevalence of clinical and subclinical mastitis by Mekuria (1986) and Shirmeka (1996).

The objective of the study was therefore:

1. To determine the prevalence of mastitis and the association of some potential risk factors in smallholder dairy private farms in and around Bahir Dar

2. To identify bacterial causes of mastitis and to conduct antimicrobial susceptibility test on the most frequently isolated ones

3. To evaluate somatic cell count (SCC) in identifying intramamary infection in local zebu and Holstein x Zebu cross breed lactating cows and determine the effect of some factors assumed to vary SCC.

2. LITERATURE REVIEW

2.1. Definition of Mastitis

When studying the literature on mastitis, difficulties are constantly encountered because the concepts "normal", "udder infection", "subclinical" and acute mastitis are insufficiently delineated.

According to Schalm *et al*. (1971) the term "mastitis" is derived from the Greek word mastos meaning breast and the suffix "itis" meaning inflammation of. Thus, mastitis means inflammation within the mammary gland. Detailed and comprehensive definition of mastitis is given by Faull and Hughes (1985) as:

Normal quarter is a quarter with no pathogens and few neutrophils in the milk and which feels normal.

Subclinical mastitis a quarter with pathogens and many neutrophils in the milk, but the milk looks normal and the quarter feels normal.

Clinical mastitis:

Acute mastitis is when there are obvious signs of inflammation of the udder such as heat, pain and swelling. The milk is macroscopically abnormal and the animal may have feverish temperature.

Subacute mastitis is when there are no obvious changes in the udder but when there are persistent clots especially in the foremilk.

2.2. Aetiology

Most commonly, mastitis begins as a result of penetration of the teat duct by pathogenic bacteria (Jabb and Kennedy, 1993). However, some viral diseases like Pseudocowpox, Herpes Mamillitis, Cowpox, Papilloma, Foot-and-Mouth disease and Vesicular Stomatitis

affecting the epithelium of the teat orifice are mentioned to result in or predispose to mastitis (Hillerton *et al*., 2001). Although variation exists on the type and isolation rate of mastitis pathogens from country to country, the most commonly incriminated and reported causes of mastitis include S*taphylococcus aureus, Streptococcus agalactiae,* Streptococcus species other than S*treptococcus agalactiae,* coagulase negative Staphylococci species, *Escherichia coli,* Micrococcus species*,* Corynebacterium species, Bacillus species, Pasteurella species, Klebsiella species*,* Mycoplasma species and Nocardia species (Pearson *et al* ., 1979; Sears *et al.*, 1991; Aaretrup *et al.,* 1995; Miltenburg *et al*., 1996; Hussien *et al*., 1997; Wilson *et al*., 1997; Sergeant *et al*., 1998; Workineh *et al*., 2002). Wilson *et al. (*1997) collected milk samples from 108,312 dairy cows from 1991 to 1995 in New York and Pennsylvania and found that over 75% of the intramammary infections were caused by S*treptococcus agalactiae*, other Streptococcus species, *Staphylococcus aureus* and coagulase negative staphylococcus species. This result indicated that these pathogens were predominant causes of bovine mastitis.

Among the bacterial species associated with bovine mastitis, two categories are distinguished. These are major pathogens which are responsible for most sever cases of mastitis and the minor pathogens, which are rarely associated with marked leuckocytosis and clinical manifestations (Rainard and Poutrel, 1988; Radostits *et al*., 1994a)

2.2.1. Major pathogens

Agreement is not yet reached to specify the identities of major pathogens in mastitis. Usually, a combination of high prevalence, the contagious nature of intramammary infection, and costly effect per case are used to determine which are major pathogens or not (Wilson *et al*., 1997). In this group are included S*taphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis,* Mycoplasma species and *Escherichia coli* (Rainard and Poutrel, 1988; Radostits *et al*., 1994a; Wilson *et al.,* 1997).

In a study carried out in Zimbabwe by Perry *et al*. (1987) S*taphylococcus aureus* was the most frequently isolated bacterium from both clinical and subclinical mastitis. Sargeant *et al.* (1998) also reported types and isolation rates as *Staphylococcus aureus* (6.8%), S*treptococcus agalactiae* (0.7%), other S*treptococcus* species (14.1%), Coliforms (17.2%), C*orynebacterium bovis* (1.7%) and other Staphylococcus species (28.7%) from mastitic milk samples collected from 834 clinical cases. This study showed that other than major

pathogens, minor pathogens could also be a common cause of severe cases of clinical mastitis. However, according to Miltenburg *et al.* (1996) the most frequent isolates from clinical mastitis were major pathogens including *Escherichia coli* (19.6%), S*taphylococcus aureus* (14.4%), S*treptococcus uberis* (11.9%) and S*treptococcus dysgalactiae* (8.9%). Torgerson *et al.* (1992) also found *Staphylococcus aureus* to be the cause of a high incidence of clinical mastitis.

Surveys of the prevalence of the various causes of intramammary infections in cattle show remarkable similarity in different countries (Radostits *et al.*, 1994a). The predominant infection in most countries is now *Staphylococcus aureus* followed closely usually by *Streptococcus agalactiae.* A relative incidence of *Streptococcucs agalactiae*, other *Streptococci* species and *Staphylococcus aureus* of 1:1:2 is a common finding. The prevalence of an individual organism and therefore the ratio between incidence and that of other organisms depend on a number of risk factors such as herd size and quality of management, especially milking hygiene and cleanness of the accommodation.

2.2.2. Minor pathogens

Some organisms, particularly non-hemolytic coagulase negative Staphylococci (CNS) and *Corynebacterium bovis* are almost ubiquitous inhabitants of the bovine mammary gland and are regarded as part of the normal flora (Jabb and Kennedy, 1993).

There is a considerable debate as to the significance of these organisms for mammary gland and for cow productivity. Minor pathogens have been credited with maintaining a higher than normal somatic cell count (SCC) and with increasing the resistance of the colonized quarter to invasion by major pathogens. There is evidence that long-term intensive programs of teat dipping and dry cow therapy can markedly reduce the prevalence of these minor pathogens (Radostits *et al.,* 1994b) which might increase the susceptibility to these minor pathogens.

Rainard and Poutrel (1988) observed that quarters initially harboring a minor pathogen were significantly less infected by new major pathogens than uninfected quarter by minor pathogens. However, Hogan *et al*. (1988) compared rates of environmental Streptococci and Coliforms infection among quarters priorly infected with either Corynebacterium or coagulate negative Staphylococci and those not infected. He found intramammary infection rate of environmental Streptococci was 3.9 times greater in *C. bovis* infected quarter than uninfected

quarters. Similarly, the rate of environmental Streptococci infections was 2.6 times greater in quarters infected with Staphylococci species than uninfected ones. No difference was observed for coliforms.

Rainard and Poutrel (1988) suggested that to envisage infections by minor pathogens as a biological tool for controlling bovine mastitis against major pathogens, it is necessary to evaluate the economical balance between the increase in milk production resulting from a reduction in prevalence of major pathogens and of the economic loss as a result of minor pathogens.

2.3. Diagnosis

Clinical mastitis is recognized by the appearance of abnormal milk, gland swelling and /or illness. Subclinical mastitis is characterized by normal milk and hence requires indirect tests to detect.

2.3.1. Somatic cell count (SCC)

Somatic cells are composed of white blood cells (WBC) and occasionally sloughed epithelial cells. Cells found in normal cattle milk from uninfected glands include neutrophils (1.1%), macrophages (66.68%), lymphocytes (10-27%) and epithelial cells (0-7%) (Larsen, 2000). When bacteria invade and colonize the mammary gland, the macrophages respond by initiating the inflammatory response that attracts polymorphonuclear cells (PMN) in to the milk to engulf and destroy bacteria. More than 90% of SCC in infected glands is composed of neutrophils. The cells can be counted by a direct microscopic method on stained milk smears. The most commonly used automated device for rapid determination of SCC in milk samples is the fossomatic milk cell counter. This instrument stains cells with a fluorescent dye and then counts the number of fluorescing particles (Schalm *et al*., 1971).

Monitoring udder health status is an important principle of mastitis control. A regular quantitative assessment of udder health status is available through the use of SCC data. The practical use of SCC data to determine cow infection status requires the selection of a threshold level (Radostitis *et al.,* 1994b).

Dohoo and Meek (1982), however, stressed that somatic cell counts are general indicators of udder health which are subject to many factors including age, stage of lactation, season, stress and management. Mean SCC decreased markedly soon after the commencement of lactation and increased during late lactation. The basic patterns of change over lactation remain the same in health or mastitic cows (Auldist *et al.*, 1995). However, Harmon (1994) argued that marked increases in SCC are a result of cells being attracted to the mammary tissue because of a direct mediators produced during a local infection, events that do not affect udder healthy are unlikely to have a direct or dramatic effect on SCC. According to him little evidence exists other than normal diurnal variation any factor did not have a major influence on SCC in the absence of intramamary infection.

To determine specificity and sensitivity of SCC (for quarters, cow or bulk milk) several studies have been conducted. Larsen (2000) reported sensitivities ranging from 73-89% with corresponding specificities of 75-85% using threshold of 200,000 cells per ml taking culture as "gold standard". Sensitivity and specificity is affected by threshold (cut point for intramammary infection). Dohoo and Meek (1982) reported 250,000 and 300, 000 cells /ml threshold to quarter and cow, respectively. Emanuelson (1997) used a threshold of 200,000 cell/ml for cow level to monitor herd mastitis in Sweden. The thresholds for milk quality have no relation to the definition of udder health categorization. At present a threshold of 100,000 cells/ml can be assumed an internationally accepted definition of udder health (Hamann, 2003). Less than 200, 000 cells/ml for cow and less than 130,000 cells/ml for bulk tank milk were reported by Larsen (2000). To the knowledge of the reviewer, no information is available on this particular issue in Ethiopia.

2. 3. 2. California mastitis test (CMT)

The California Mastitis Test (CMT) remains the only reliable screening test for subclinical mastitis that can be easily used at the cow side (Schalm *et al.,* 1971). The CMT was developed to test milk from individual quarters but also been used on composite and bulk milk samples.

The CMT involves mixing and swirling equal parts of bromocresol violet reagent and milk in a plastic paddle with a compartment for each quarter (Quinn et al., 1999). The test results are interpreted subjectively as either a negative, trace, 1^+, 2^+ or 3^+ inflammatory response based on the viscosity of the gel formed by mixing the reagent with milk (Radostits *et al.,* 1994b).

Fresh unrefrigerated milk can be tested using the CMT for up to 12 hours. Reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used, the milk must be thoroughly mixed prior to testing because somatic cells tend to segregate with milk fat. The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. The degree of reaction between the detergent and the DNA of nuclei is a measure of the numbers of somatic cells in milk. The threshold for CMT scores depend on the objective of the study. If it is used to minimize the rate of false negatives, the test should be read as negative versus positive with trace scores regarded as/ recorded as positive. If the CMT is to be used in culling decisions, a threshold with a lower rate of false positives may be desirable (Larsen, 2000).

2.3.3. Culture

The microbiological examination of both individual cow and bulk tank culture are elements of mastitis control. Most mastitis control programs include the use of individual cow cultures to determine which mastitis pathogens are present on the farm. Culturing can be used in a targeted fashion for specific control programs such as segregation plans for contagious mastitis or for surveillance to detect the presence of new or emerging pathogen. Culturing is also used to evaluate treatment efficacy and to establish susceptibility patterns to aid in the development of rational treatment strategies (Larsen, 2000).

There have been a number of studies to improve culture quality in identification of intramamary infection. Comparisons were made on pre and post milking samples, pre culture incubation, pre culture freezing, increased plate inoculation volumes, frequency of sampling and centrifugation (Dinsmore *et al*., 1992).

Sears *et al.*(1991) using both pre-milking and post-milking positive results as definitive diagnosis ("gold standard"), found sensitivities of 92, 86 and 99% for S*taphylococcus aureus,* coagulase negative Staphylococci and for Streptococcus species other than S*treptococcus agalactiae* in pre-milking milk samples, respectively. Similarly, for post-milking samples the corresponding values were 96, 98, and 99%. The sensitivity was higher in pre-milking samples although multiple isolates were more common. This study suggested that unless specific advantages can be demonstrated for pre-milking samples, collection of post-milking samples is recommended in order to minimize the likelihood of contamination.

Dinsmore *et al.* (1992) compared pre-culture incubation, pre-culture freezing and increased plate inoculation volumes with standard culture techniques. They found that pre-culture incubation and larger plate inoculation volumes (0.1ml) significantly increased the recovery rate of bacterial pathogens over the standard culture method. Recovery was enhanced significantly by this method for several organisms including environmental Streptococci and Coliform bacteria. Freezing milk before culture yielded a significantly higher positive culture rate. Sensitivity of bacterial culture could be increased by including second and third consecutive samples as this overcomes the problem of cyclic shedders (Sears *et al.,* 1993). The most widely accepted criterion for the diagnosis of intramamary infection is that it exists when the same organism is isolated from two samples or two of the three consecutive samples taken every other day.

2.4. Control of Bovine Mastitis

2.4.1. Mastitis therapy

Because of the diverse bacterial etiologies of the disease a variety of control methods involving hygiene prior to, during and after milking are used to minimize exposure of cows to mastitis organisms. Despite these procedures, new cases of mastitis invariably occur and antimicrobial therapy plays a role in the control of bovine mastitis (Owens *et al.,* 1997).

The major obstacle in treating mastitis is antibiotic resistance. Watts and Salmon *(*1997) conducted a study to see the activity of antimicrobial agents against *Staphylococcus aureus* strains from intramamary infections (70 β lactam positive, and 37 β - lactam negative, a total of 107 *Staphylococcus aureus* strains). They found that penicillin and ampicillin to be most affected by β - lactamase activity but oxacillin, cephapirin and ceftiofur were not affected. In this study, penicillin plus novobiocin demonstrated good activity against both strains positive and negative for β -lactamase. However, Sol *et al.* (1995) reported resistance of *Staphylococcus aureus* strains to penicillin not to be the main factors associated with bacteriological cure, rather age of the cow, SCC at the time of treatment, and stage of lactation were mentioned to be the most important factors associated with cure. Owens *et al*. (1997) found *S. aureus* and Streptococci species to be susceptible to penicillin and novobiocin combination. This result indicated susceptibility that tests of individual compounds might not

predict susceptibility to the combination of these compounds. In Ethiopia, Hussein *et al*. (1997) reported, out of five isolates of S. *aureus* four were sensitive to penicillin and streptomycin, all to erythromycin, three to chloramphenicol and ampicillin but most isolates (four) were less sensitive to tetracycline.

2.5. Epidemiology of Bovine Mastitis in Ethiopia

2.5.1. Prevalence

Kassa *et al.* (1999) carried out a survey of mastitis in dairy herds of the Ethiopian central high lands. Out of 10, 908 quarters examined from 2,681 cows, they found prevalence of clinical mastitis, non-functional or blocked quarters and subclinical mastitis to be 1.2%, 3.8% and 38.9% on cow basis, respectively. According to Hussein *et al.* (1997) the prevalence of clinical and subclinical mastitis are found to be 5.3% and 19% on cow basis and 1.9% and 7.4 % on quarter basis, respectively, in the central regions of Ethiopia. In a study conducted at Repi and Debre-Zeit dairy farms, out of 186 lactating cows, forty (21.5%) were clinically affected and 71 (38.%) subclinically infected (Workineh *et al.,* 2002). The overall prevalence in this study was 59.7%. In another investigation by Lemma *et al.* (2001), of major diseases of crossbred dairy cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases, in which 171 cows out of 556 were found to be affected. Bishi (1998) reported mastitis prevalence rates of 34.3% and 5.3% at cow level in Addis Ababa region, for subclinical and clinical mastitis, respectively. In the same study area, Mungube (2001) reported an overall prevalence of 46.6% for subclinical mastitis at cow level and 27.8% at quarter level. This great variation could result from differences in environment and management (Kerro, 1997).

2.5.2. Incidence

The information on this issue is scant as monitoring activities are not well organized. However, a survey on bovine mastitis in milking cows at Alemaya University Dairy Farm by Tefera (2001) for 6 years (from 1993 to 1998) revealed an overall clinical mastitis incidence of 34 cases/100 cows per year.

2.5.3. Economic loss

In Ethiopia, there is limited information on the economic loss due to mastitis. However, the few data available indicate that the loss is significant. Mungube (2001) estimated the economic loss from mastitis in the urban and periurban areas of Addis Ababa. He used milk production losses, treatment costs, withdrawal and culling losses as parameters for calculating losses. This loss was found to be 210.8 birr per cow per lactation. In this study, loss due to culling, milk loss, treatment, and withdrawal contributed 49%, 38.4%, 9.3% and 3.3% to the total mastitis losses, respectively. Milk production losses contributed 38.4% of the total losses, sub clinical mastitis contributing 94% and clinical mastitis 6% of the milk losses. Sub clinical mastitis contributed 36.1% of the total losses, which is primarily due to, reduced milk production. Bishi (1998) also reported the economic losses from clinical and subclinical mastitis to be approximately 270 Ethiopian birr per cow per lactation.

3. MATERIALS AND METHODS

3.1. Study Area

Bureau of Agriculture of Amhara Regional State identified three potential milk production areas based on market availability, comparative advantage, biophysical potential and other socioeconomic parameters. These areas are big towns and surrounding districts within a radius of 150 km. The potential areas identified are Bahir Dar, Dessie and Debre Berhan milk shedes. This study was conducted in one of these areas, the Bahir Dar Milk Shed which comprises of Bahir Dar town and two other surrounding districts, Woreta and Adet. While Bahir Dar is located 567 Km northwest of Addis Ababa, the capital city of Ethiopia, Woreta and Adet are located nearly 50 Km northeast and southeast of Bahir Dar, respectively (BoA, 2003).

Bahir Dar is located at an altitude of 1802 a. b. s. and has a warm humid climate with an average annual rainfall of 700 mm. The annual mean temperature ranges from 12.4 - 27 ^{0}C (CSA, 2001).

3.2. Study Population

According to the recent census (CSA, 2003) the total cattle population of Amhara region is estimated to be 10,512,777. Out of this population, the male cattle constitute about 50.2% (5,273,390); the remaining 49.8% (5,239,386) are female cattle. The majority of cattle population is found in rural areas, while a very small proportion is accounted for urban areas (1.5%). Except few hybrid (0.4%) and exotic (0.1%) animals, the majority (99.5%) of cattle population in the region are local breeds. According to a study conducted in Bahir Dar milk shed by Addisu and Zelalem (2003), the number of dairy cows in Bahir Dar milk shed is estimated to be 3301 out of which 1228 (37.2%) are crossbreds and 1633 (62.8%) are local breeds. The total number of milking cows are 1633 (49.46%) out of which 697 (42.68%) are crosses and the remaining 936 (57.31%) are locals. The study animals were lactating local zebu and Holstein x Zebu crossbreds and were selected from the above indicated population.

3.3. Study Design

3.3.1. Prevalence study

Prevalence of mastitis was determined cross sectionally from September 2003 to March 2004 in and around Bahir Dar at cow and quarter level based on clinical manifestations for clinical prevalence and indirect tests (CMT and culture) for subclinical prevalence. Prevalence was calculated according to the formula given in Thrusfeild (1995).

$$\text{Prevalence} = \frac{\text{No. of animals with the disease}}{\text{No. of animals at risk}}$$

3.3.1.1. Sample size determination and sampling strategy

The sample size was determined at 95% confidence interval, 5% precision and from previous studies in the study area (Shirmeka, 1996), with an expected prevalence of 40%. Thus, the sample size value was read from Thrusfeild (1995) sample size Table to be 369 animals. Simple random sampling was considered to select the animals, assuming the difference in clusters (households in this study) was minimal when the herd size was so small (two in this study) (Martin *et al*., 1987). To select these cows, neither list of lactating cows (sampling frame) nor a household was found in the study area. It was difficult to record all lactating cows; therefore, the household or owners list was recorded by employing data collectors and was taken as a sampling frame. The number of households to be selected was determined by dividing sample size with herd size, in this case 369/2= 184. However, due to lack of cooperation by some owners only 163 households or 351 lactating cross and local zebu breeds, less by 18 were included in the investigation.

3.3.1.2. Definition

This definition was according to International Dairy Federation recommendations, IDF (1987).

Clinical mastitis:

A cow or a quarter was considered clinically sick for mastitis when abnormality was observed in milk (like the presence of flakes, clots, bloody or watery appearance), in the udder (like swelling, pain, hotness) or in the cow (systemic signs together with the above manifestations).

Subclinical mastitis:

A cow or a quarter was considered to have subclinical mastitis intramamary infections if CMT score was 1, 2, 3 or when a mastitis pathogen was isolated.

3.3.1.3. Data collection

Questionnaire was compiled to evaluate the effect of potential risk factors on the occurrence of mastitis and somatic cell count (SCC). Data on each sampled cow was collected in a properly designed format (Annex 1). Risk factors considered were breed, parity, stage of lactation, quarter location and presence of tick and or lesion on the udder skin or teat. The factors were categorized into host factors, management and environmental factors

3.3.1.4. Milk sample collection

Milk sample collection was according to the procedures recommended by National Mastitis Council, NMC (1990). To avoid the effect of time between milking and sampling on SCC, milk sample was collected before milking early in the morning. As SCC dictate immediate processing, to avoid possibility of contamination for culture during milk film preparation for SCC, duplicate quarter foremilk samples of approximately 10ml amount was taken; one sample was used for CMT and SCC and the remaining sample used for bacterial isolation. After collection, samples were placed in icebox and processed in the same day or within few days. Milk collection procedure is described below.

Preparing udders and teats

The udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with a pledged of cotton moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing, was avoided by scrubbing, the teats on the far side of the udder first, then those on the near side. Separate pledged cotton was used for each teat.

Collecting milk samples

Teats towards sample collection were sampled first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was held as near horizontal as possible, and by turning the teat to a near horizontal position, approximately 10 ml of milk was collected into a universal sample collection bottle.

After collection, the sample was placed in icebox and transported to the laboratory. The samples were either cultured or stored at 4 °C until cultured within few days.

3.3.1.5. CMT screening

The California Mastitis Test was carried out according to the method described by Quinn *et al*. (1999).

A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 seconds. The result was scored from 0-3 and the interpretation is presented in Annex 2.

3.3.2. Bacterial isolation and antibiotic susceptibility test

3.3.2.1. Bacterial isolation

Bacteriology was performed on all quarter foremilk samples regardless of their CMT and SCC values to use culture results as a gold standard to evaluate SCC. Out of 1404 quarters, 54 were found blocked and three samples were lost during handling, hence, milk samples were collected from 1347 functional quarters and cultured.

Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in National Mastitis Council, NMC (1990).

Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25^0C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently. One standard loop (0.01ml) of milk sample was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37 ^{0}C. The plates were checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as Corynebacterium species. For primary identification, colony size, shape, color, hemolytic characteristics, Grams reaction and catalase production were used. This was conducted at Bahir Dar Regional Veterinary Laboratory, Bahir Dar. After primary identification, isolates were transported in Brain Heart Infusion transport medium to the Faculty of Veterinary Medicine, Debre Zeit for confirmation. For confirmation, biochemical tests were used after subculturing isolated distinct colony on to a nutrient agar. The procedures followed for the identified pathogens is presented in Annex 3.

Interpretation was made according to National Mastitis Council, NMC (1990). The culture was considered negative if no growth occurs after 72 hours of incubation. Isolation of two or more colonies from a quarter sample was considered contaminated and the result was disregarded. However, isolation of *S. aureus* and *Str. agalactiae* from contaminated sample was considered as causal.

3.3.2.2. Antibiotic susceptibility test

Eighty-one isolates after checking for purity were tested for susceptibility to selected antibiotics. The isolates were 24 *S. aureus*, 10 *Str. agalactiae*, 9 *Str. dysgalactiae*, 2 *Str. uberis*, 22 coagulase negative Staphylococcus species (CNS), 7 Micrococcus species, 5 *C. bovis* and *2 B. cerus*. Antimicrobials used in this study were sulfisoxazole, tetracycline, erythromycin, oxacillin, chloramphenicol, clindamycin and streptomycin. The selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability. Representative was taken for those antibiotics for which prediction is possible by the result of a representative (that is individual members within the group are related closely enough to assume cross-resistance). Tetracycline, sulfisoxazole, erythromycin and clindamycin were used as a representative to predict the result against all other tetracyclines, sulfonamides, macrolids and lincomycin, respectively.

Agar disc diffusion (Kirby - Bauer method) was used as described in Quinn *et al*.(1999). The procedures for the preparation of inoculum, inoculation to the Mueller – Hinton agar and disc application are presented in Annex 4. For Streptococcus species and *C. bovis* blood was added to Mueller – Hinton agar . After measuring the zone of inhibition, isolates were classified sensitive, moderately sensitive, intermediate and resistant. National Committee for Clinical Laboratory Standard (NCCLS) breakpoints was used to interpret the inhibition zone adapted from in Quinn *et al.* (1999).

Retrospective data were compiled on the type of antibiotics used to treat mastitis and other infectious diseases in the region. Specifically the antibiotics used to treat clinical mastitic cases from August 1999- March 2004 were gathered from clinical casebook records.

3.3.3. Establishing somatic cell count (SCC) threshold level

To establish a SCC threshold level that is used to classify a quarter free of intramamary infection or not, culture was used as a gold standard and hence all milk samples irrespective of their CMT score and SCC value were cultured (Erskine, 2001). Those quarters which were culture negative were considered ‘healthy’. The mean SCC value of these quarters which

were classified as 'healthy' on culture results was taken as a reference SCC threshold level. Considering the assumption of Thrusfeild (1995) to define cutoff points for continuous tests ten to twenty thousand cells/ml standard deviation above and below the reference SCC threshold level (mean SCC of 'healthy' quarters) values were evaluated against culture to identify infected quarter. Their specificity and sensitivity was computed using Winepiscop 2.0 software. The ten SCC threshold values(x 10^3) considered for this study for crossbreds were 100, 120, 150, 170, 190, 200, 210, 230000, 250 and 300 cells per milliliter of milk and for local zebu threshold values were 80, 100, 120, 150, 170, 190, 200, 210, 230 and 250 cells/ml.

The selection of the threshold value or critical value depends on the element of the test that is most important to optimize (Erskine, 2001). In this study, threshold levels which enhance test sensitivity were identified from computed values and this would ensure as many infected cows as possible. Similarly, a level that increased specificity was also identified. It is up to the farmer to choose the level that best serves his or her purpose. For example, for dry cow therapy the farmer will choose a level which will increase sensitivity and for culling decisions high specificity levels will be the best choice. This study identified these two values for local zebu and Holstein x Zebu breeds. Threshold level was determined independently for each breed as it was found in this study, breed to affect SCC significantly.

In the absence of gold standard or for cost or availability reasons, available tests will be used in combination parallely or serially to increase the validity of the test (Thrusfeild, 1995). In this study, different levels of SCC were evaluated when used in combination with CMT. This was done after sensitivity and specificity was determined for each test with the gold standard culture and was computed with Winepiscoe 2.0 software. This was done on Holstein x Zebu crosses taking as an example that combination of CMT and SCC improve the quality of the test.

3.3.3.1. Preparation of milk films, staining and counting of somatic cells

For counting somatic cells, microscopic method was used. Milk film preparation, staining and counting were done according to the standards set by International Dairy Federation (IDF, 1995). To obtain a uniform distribution of cells, milk samples were mixed by moving up side down gently 25 times and allowed to stand for 2 minutes to permit air bubbles and foam to disappear. Microscopic slides were degreased with alcohol before milk film preparation. A

0.01ml of milk was taken with a 50µl micropipette calibrated at 10 and spread evenly over one cm^2 area on a microscopic slide and allowed to dry at room temperature on a leveled table. One cm^2 area was delineated by a template prepared from a cap board. Dried films were dipped in Levowrtz-Webers modification dye for 30 minutes. Levowrtz- Webers modification dye was prepared according to IDF (1995) recommended procedures. After 30 minutes, slides were left to dry for a few minutes, washed with tap water then allowed to dry again in a dust free area. Stained slides were stored in slide box until counted. Using oil immersion objective those cell nuclei clearly recognizable and those at the periphery with more than 50% of the cell body in view were counted. Fifty fields were counted from each sampled quarter.

The number of cells per ml of milk was calculated by multiplying the average number of cells per field with MF

$$MF = \frac{40{,}000}{3.1416 * d^2}$$

Where MF= magnification factor

d= diameter

To measure the diameter, first stage micrometer slide was placed on a microscope stage. Then under oil immersion objective the number of divisions in stage micrometer were counted. Each division on a stage micrometer slide represents 0.01mm and hence to calculate the field diameter, the number of divisions counted were multiplied by 0.01mm.

3.4. Statistical Analyses

Percentage with confidence interval was used for prevalence determination and calculated with Stata 7.0 software.

Factors that affect the prevalence of mastitis and SCC included in this study were breed, stage of lactation, parity, quarter location and presence of tick and/or lesion on udder or teat. For SCC in addition to aforementioned factors, the effect of infection status was also evaluated. In case of prevalence of mastitis, factors were first screened by running univariate logistic regression between each risk factor and occurrence of mastitis then risk factor with a p value less than 0.25 were further analyzed in multiple logistic regression and two way ANOVA in

case of SCC was used. For SCC statistical test was carried out on Log_{10} transformed SCC data to fulfill assumption of normality. The analysis was run with Stata 7 software (Annex 5).

In data entry, stage of lactation and parity were categorized in to three levels and coded 1 to 3. For stage of lactation 1 represent, 1-120 days post partum (early lactation), 2 from121-240 days (middle lactation) and 3 represent days greater than 240 (late lactation). For parity 1, 2 and 3 represent, respectively, 1-2, 3-5 and greater than 5 lactation numbers. Infection status of the quarter was also categorized in to three levels and was denoted as 0, 1 and 2 representing no infection, presence of minor pathogens and major pathogens in the respective order. The other risk factors were denoted 1 and 0 where 1 indicate the presence and 0 absence of risk in the hypothesis.

Arithmetic mean was used to calculate SCC mean for local zebu and crossbreds of uninfected quarters and t-test was used to evaluate whether significant difference occurs between these two breeds.

Significant factors with three levels in SCC and in occurrence of mastitis were compared using t-test and chi square, respectively, to identify in which level the difference exists.

For sensitivity and specificity calculation of the threshold level, Winepiscope 2.0 software was used.

4. RESULTS

4.1. Prevalence and Risk factors

4.1.1. Prevalence

A total of 351 lactating cows (195 indigenous zebu and 156 Holstein x Zebu) in private smallholder dairy farms in Bahir Dar milk shed were investigated from September 2003 to March 2004 cross sectionally to determine the magnitude of mastitis. Out of 1404 quarters examined, 54 (3.8%) were blocked. Clinical prevalence at cow level was 3.9% in crossbreds and none in local zebu breeds (Table 1).

Table 1. Prevalence of clinical mastitis at cow level in local zebu and cross breed lactating cows in Bahir Dar milk shed

Breed	*No. animals examined*	*Status of clinical mastitis*		
		positive	negative	prevalence (%) and 95% CI
Cross	154	6	148	3.89 (95% CI 0.82 - 7.17)
Local zebu	195	0	195	0
All animals	349	6	343	1.71 (95% CI 0.34 - 3.08)

Subclinical mastitis at cow level was 34.4% in crossbreds and 17.9% in indigenous zebu using CMT (Table 2). Based on culture subclinical mastitis prevalence at cow level was 30.5% in crossbreds and 12.8% in indigenous zebu (Table 3).

Table 2. Prevalence of subclinical mastitis at cow level using CMT in local zebu and crossbred lactating cows in Bahir Dar milk shed

Breed	*No. of animals sampled*	*Status of subclinical mastitis*		
		positive	negative	prevalence (%)
Cross	154	53	101	34.41 (95% CI 27.59 - 43.07)
Local zebu	195	35	160	17.94 (95% CI 12.18 - 22.81)
All animals	349	88	261	25.14 (95% CI 20.57 - 29.71)

Table 3. Prevalence of subclinical mastitis at cow level using culture in local zebu and crossbreed lactating cows in Bahir Dar milk shed

Breed	*No. of animals sampled*	*Status of subclinical mastitis*		
		positive	negative	prevalence (%)
Cross	155	47	108	30.52 (95% CI 23.16 - 37.87%)
Local zebu	195	25	170	12.82 (95% CI 8.00 - 17.55)
All animals	350	72	278	20.63 (95% CI 16.00 - 24.89%)

Based on CMT and culture, the prevalence of quarter level subclinical mastitis in crossbreed and local zebu cows was 17.9% and 4.9% and 14.7% and 6.6%, respectively (Table 4 and Table 5)

Table 4. Prevalence of subclinical mastitis at quarter level using CMT in local zebu and cross breed lactating cows in Bahir Dar milk shed

Breed	*No. of quarters sampled*	*Status of subclinical mastitis*		
		positive	negative	prevalence (%)
Cross	564	101	463	17.90 (95% CI 14.73 - 21.08)
Local zebu	747	37	710	4.95 (95% CI 3.39 - 6.51)
All animals	1311	138	1173	10.52 (95% CI 8.86 - 12.18)

Table 5. Prevalence of subclinical mastitis at quarter level using culture in local zebu and crossbreds

Breed	*No. of quarters sampled*	*Status of subclinical mastitis*		
		positive	negative	prevalence (%)
Cross	563	83	480	14.74 (95% CI 11.80 - 17.67)
Local zebu	772	51	721	6.60 (95% CI 4.85 - 8.36)
All animals	1335	134	1201	10.04 (95% CI 8.42 - 11.65)

Both at cow and quarter level, prevalence was high in crossbreds compared to locals. The difference was statistically significant ($p < 0.05$).

4.1.2. Risk factors affecting prevalence of mastitis

Five factors were considered as potential risks for the occurrence of subclinical mastitis in this study. These were breed, stage of lactation, parity, quarter location and presence of tick and/or lesion on udder or teat. In a univariate logistic regression analysis, breed and stage of lactation were found to be significant ($p < 0.05$, Table 6) (Annex 5).

Table 6. Univariate logistic regression of risk factors for the occurrence of subclinical mastitis

Variable	*Odds ratio*	*p value*
breed	3.140831	0.0000
stage of lactation	1.815218	0.000
parity	1.39136	0.051
presence of tick and/or lesion	1.674234	0.121
quarter location	1.027564	0.899

Risk factors with a p value less than 0.25 were further considered in multiple logistic regression analysis. Breed, stage of lactation and parity were significant ($p < 0.05$, Table 7). For stage of lactation significant difference was observed between late and early stage of lactation (chi =28.5, df =1, $p = 0.000$), however, no difference was observed between early and middle, and middle and late stage of lactations ($p > 0.05$). The occurrence of subclinical mastitis was high in late stage of lactation. Similarly, significant difference in all possible combinations for parity was observed ($p < 0.05$). Differences between parity number 1 to 2 (coded as 1 in statistical analysis part) and parity 3 to 5 (chi =6.6, df =1, $p = 0.010$), parity 1 to 2 and greater than 5 (chi =20.4, df =1, $p = 0.000$) and parity 3 to 5 and greater than 5 (chi 4.5 df =1, $p = 0.033$) were significant. The occurrence of subclinical mastitis was high in parity 3 to 5.

Table 7. Risk factors of bovine subclinical mastitis significant in a final multiple logistic regression module

Variable	*Odds ratio*	*p value*
breed	3.091657	0.000
stage of lactation	1.928416	0.000
parity	1.577942	0.000

The prevalence (quarter prevalence using CMT) in crossbreds was 17.9% and 4.9% in local breeds. This difference in breed was statistically significant ($p < 0.05$). The occurrence of mastitis was three times more likely in crossbreds compared to local zebu breeds (Odds ratio=3.09, Table 7).

4.2. Bacterial Isolates and Antibiotic Susceptibility Test

4.2.1. Bacterial isolates

Milk sample was collected from 1347 quarters coming from 351 (195 lactating local zebu and 156 cross breed) lactating cows. Of all quarter foremilk samples, 135 (10%) samples were culture positive (single colony), 12 (1%) showed mixed growth and 1200 (89%) yield no bacteria. In this study, coagulase negative Staphylococci (CNS) species were the most predominant pathogens constituting 49.6% of all isolates followed by *S. aureus* (17.8%), *Str. agalactiae* (8.2%) and *Str. dysgalactiae* (6.7%) (Table 8).

Table 8. Frequency distribution of bacterial isolates from mastitic quarter foremilk samples of local zebu and cross breed lactating cows in Bahir Dar milk shed

No.	*Isolate*	*frequency*	*Percentage*
1	*S. aureus*	24	17.8
2	*Str. agalactiae*	11	8.2
3	*S. intermedius*	7	5.2
4	CNS*	67	49.6
5	Micrococcus species	7	5.2
6	*Str. dysgalactiae*	9	6.7
7	*Str. uberis*	2	1.5
8	*C. bovis*	5	3.7
9	*A. pyogens*	1	0.7
10	*B. cereus*	2	1.5
Total		**135**	**100%**
Mixed growth		12	
No growth		1200	
	Total	1347	

*CNS = Coagulate negative staphylococci

The prevalence of bacterial pathogens in local zebu and crossbreed lactating cows is shown in Table 9. The proportion of major and minor pathogens in local zebu breeds was 41.2% and 58.8%, respectively; and in crossbreds, it was 38.0% and 61.9% in that order. S*taphylococcus aureus, Str. agalactiae, S. intermedius, Str. dysgalactiae, Str. uberis* and *A. pyogens* were included under major pathogen. Under minor pathogens were CNS, Micrococcus species, *C. bovis* and *B. cereus*. The proportion of minor pathogens was high (61.9%) in cross breeds compared to local zebu (58.84%). Coagulase negative Staphylococci were found dominant and isolated in comparable proportions in the 2 breeds, 47.05% in local zebu breeds and 51.19% in crossbreds.

Table 9. Frequency distribution of bacterial isolates from mastitic milk in local zebu and cross breed lactating cows in the Bahir Dar milkshed (2003-2004)

No.	*Isolate*	*Local breed*		*Crossbred*		*Total*
		frequency	%	frequency	%	
	Major pathogens					
1	*S. aureus*	10	19.60	14	16.67	24
2	*Str. agalactiae*	1	1.96	10	11.90	11
3	*S. intermedius*	3	5.88	4	4.76	7
4	*Str. dysgalactiae*	6	11.76	3	3.57	9
5	*Str. uberis*	1	1.96	1	1.19	2
6	*A. pyogens*	0	0	1	1.19	1
	Minor pathogens					
1	CNS*	24	47.05	43	51.19	67
2	Micrococcus species	0	0	7	8.33	7
3	*C. bovis*	4	7.84	1	1.19	5
4	*B. cereus*	2	3.92	0	0	2
Total		51	100	84	100	135

*CNS = *Coagulate negative staphylococci*

The majority of the isolates in this study were from subclinical mastitis. Out of 11 quarters affected clinically, bacteria were isolated from three quarters only; the remaining eight yielded no bacteria. Isolates from clinical cases were CNS, *Str. agalactiae* and *Str. dysgalactiae.* Out of 54 blocked quarters, six were from clinically affected cows meaning one in each clinical cow indicating clinical mastitis will hamper future productivity of cows.

4.2.2. Antibiotic susceptibility results

A total of 81 isolates including 24 (29.6%) *S. aureus*, 22 (27.2%) CNS, 10 (12.3%) *Str. agalactiae*, 9 (11%) *Str. dysgalactiae*, 7 (8.6%) Micrococcus species, 5 (6.2%) *C. bovis*, 2 (2.5%) *Str. uberis* and 2 (2.5%) *B. cereus* were tested for susceptibility to seven antibiotics.

The antibiotics were sulfisoxazole, tetracycline, erythromycin, oxacillin, chloramphenicol, clindamycin and streptomycin.

When comparing the overall efficacy (on all isolates), sulfisoxazole was the most effective antibiotic where 96.3% of the total isolates were found susceptible. Following sulfisoxazole were erythromycin and clindamycin where effective on 95% of the total isolates. Tetracycline and chloramphenicol were also effective whereby 91.4% and 86.4% of the total isolates were susceptible, respectively. The least effective drug was streptomycin where only 60.5% of the total bacterial population were susceptible. Oxacillin was also with relatively weak efficacy whereby 75.4% of the total isolates were susceptible.

In the present study, CNS isolates were more susceptible to sulfisoxazole (95.5%), tetracycline (90.9%), erythromycin (95.5%), clindamycin (90.9%), and streptomycin (81.8%) and resistant to chloramphenicol and oxacillin with isolates having susceptibilities of only 68.2% and 68.2%, respectively. Hence, sulfisoxazole, tetracycline and erythromycin are the drug of choice for CNS. *Staphylococcus aureus* isolates were most susceptible to sulfisoxazole (100%), chloramphenicol (100%), clindamycin (95.8%) and erythromycin (95.8%). Oxacillin and tetracycline were also effective on *S. aureus* and showed 91.7% and 89.3% susceptibility percentages, respectively. *Staphylococcus aureus* showed resistance to streptomycin whereby only 29.2% of the total isolates were susceptible. In this study, tetracycline and chloramphenicol were effective on *S. aureus* isolates. From Streptococcus species *Str. agalactiae* and *Str. dysgalactiae* showed highest resistance to streptomycin (100% resistance). *Streptococcus dysgalactiae* isolates were also only 44.4% sensitive to oxacillin. These two species were the most frequently isolated pathogens among Streptococcus species and were susceptible to sulfisoxazole, tetracycline, erythromycin, chloramphenicol and clindamycin. Generally, in this study streptomycin showed very poor efficacy in almost all isolates. Oxacillin was also weak in some isolates like Streptococci species (Table 10).

Table 10. Results of antibiotic susceptibility test on bacteria isolated from milk samples obtained from cows with mastitis

No.	Drug Name	*S. aureus* (24 **)	*Str. agalactiae* (10)	*Str. dsysgalactiae* (9)	*Str. uberis* (2)	CNS (22)	Micrococcus Species (7)	*C. bovis* (5)	*B. cerus* (2)
1	Sulfisoxazole	100%*	100%	88.89%	100%	95.46%	85.80%	100%	100%
2	Tetracycline	87.50%	100%	88.89%	100%	90.91 %	100%	80%	100%
3	Erythromycin	95.84%	90%	88.89%	100%	95.46%	100%	100%	100%
4	Oxacillin	91.67%	77.78%	44.44%	100%	68.19%	85.72%	20%	50%
5	chloramphinicol	100%	100%	100%	100%	68.19%	85.72%	80%	100%(2/2)
6	Clindamycin	95.84%	100%	100%	100%	91%	100%	80%)	100%
7	Streptomycin	29.2%	0%	0%	100%	81.82%	100%	80%	50%

* = %, percentage of susceptible isolates ** = numbers in bracket indicate the total number of isolates tested

4.2.2.1 Frequently used antibiotics to treat cases of mastitis and other infectious diseases in Bahir Dar milk shed

A five year retrospective data (from 1999 - 2004) obtained from clinical records in the study area showed that there was a tradition of using specific drugs. The types of antibiotics used in the study area were pen-sterp (penicillin and streptomycin combination), oxytetracycline, intramamary infusions, penicillin and intertrium (trimethoprim and sulfonamide combination). Pen-strep (penicillin and streptomycin combination) and oxytetracycline were the most widely used drugs to treat mastitis and other infectious diseases (Table 11). Sulfonamides were the least frequently used drugs to treat cattle.

Table 11. Mastitis cases and type of drugs used for therapy in Bahir Dar Veterinary Clinic (1999 – 2004)

No.	*Commercial Name*	*Common Name*	*No.of masstitic cases treated*	*%*
1	Pen- Strep	Procanine penicillin BP 200mg and Dihydrostreptomycin BP 250mg	127	69.78
2	Alamycin LA	Oxytetracycline dihydrate 200mg	4	2.19
3	Procaine penicillin	Procaine penicillin 4 million IU	7	3.84
4	Procaben	Procaine penicillin and benzathine penicillin	5	2.04
5	Benzanthine penicillin	Benzantine penicillin	10	6.49
6	Intramamary infusion	Predisolone Procaine penicillin Dihydrostreptomycin	2	1.09
7	Mastitis injector	Procaine penicillin G 100,000 IU Streptomycin sulphate 100 mg Neomycin Sulphate 100 mg Prednisolone 10 gm	9	4.94
8	Oxytetracyclin e 20%	Oxytettracycline hydrochloride 100mg Predisolone	15	8.24
9	Norocillin	Procaine penicillin 300 mg	3	1.64

4.3. Somatic cell count (SCC)

4.3.1. Somatic cell count (SCC) threshold level

The SCC of all milk samples collected from all quarters of mastitis and non-mastitic cows were counted to determine the threshold level for local zebu and cross breed cows. The mean, minimum and maximum values of apparently healthy quarters for both breeds are shown in Table 12. The maximum SCC for both breeds were close to a million though that of cross breeds was higher. This difference was significant ($p < 0.05$) and therefore threshold level was determined for each breed independently.

Table 12. Mean SCC of uninfected quarter ('healthy' quarter) of cross and local breed cows

Breed	*variable*	*Mean*	*Std Dev.*	*Std. Err.*	*95% CI*	*Min*	*Max*
Cross	SCC	168,944.8	196,146.7	13,855.68	145,696.7-200,317.4	7859	984,666
Local	SCC	130,887.6	135,448.3	5,213.40	120,651.1-141,124	0	905,412

4.3.1.1. Threshold level for crossbreds

The sensitivity, specificity, positive predictive value and negative predictive value of SCC was determined at 100000, 120000, 150000, 170000, 190000, 200000, 210000, 230000, 250000 and 300000 cutoff points taking culture result as a gold standard. Calculation was performed using Winepiscoe 2.0 software. High sensitivity which detects as many infected cows as possible was found at100, 000 cell/ml among the considered cutoff points in the current study (Table 13). Similarly, high specificity was observed at 300,000 cell/ml (Table 13). Increasing specificity or sensitivity was at the expense of one another. The difference between the two, narrowed at threshold of 300,000 cell/ml where sensitivity and specificity was 79.16 and 80.28%, respectively.

Table13. Sensitivity and specificity values of SCC for cross breed cows at ten different cutoff points taking culture as a gold standard

Threshold /cutoff point cell/ml	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
100,000	95.80	45.52	23.23	98.45
120,000	89.58	54.48	25.29	96.81
150,000	87.50	64.87	30.00	96.79
170,000	87.50	68.46	32.30	96.95
190,000	85.41	71.68	34.16	96.61
200,000	83.33	72.04	33.89	96.17
210,000	81.25	73.83	34.82	95.81
230,000	79.16	76.70	36.89	95.53
250,000	79.16	77.41	37.62	95.57
300,000	79.16	80.28	40.86	95.72

4.3.1.2. Threshold level for local breeds

Similar to crossbreds sensitivity, specificity, positive predictive value and negative predictive value of SCC was determined at 80000, 100000, 120000, 150000, 170000, 190000, 200000, 210000, 230000 and 250000 cutoff points taking culture result as a gold standard. Higher sensitivity and specificity among the cutoff points was observed at 80,000 cell/ml and 250,000 cells/ml with values of 88.23% and 85.15%, respectively (Table 14). The difference between sensitivity and specificity narrowed at threshold of 150,000 cell/ml with values of 67.64% and 71.47%, respectively.

Table 14. Sensitivity and specificity values of SCC for local zebu at ten different cutoff points taking culture as a gold standard

No.	*Threshold /cutoff point cell/ml*	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
1	80,000	88.23	45.82	7.38	98.75
2	100,000	82.35	51.72	7.73	98.35
3	120,000	73.52	62.24	8.71	97.95
4	150,000	67.64	71.47	10.40	97.83
5	170,000	64.70	75.48	11.70	7.10
6	190,000	61.76	77.25	12.00	97.57
7	200,000	61.76	78.67	12.42	97.67
8	210,000	61.76	81.24	14.18	97.69
9	230,000	58.82	83.60	15.26	97.58
10	250,000	58.82	85.15	16.26	97.68

4.3.2.3. Threshold for SCC when used in combination with CMT (the case of crossbreds)

Sensitivity increased at 100,000 cells/ml when used in combination with CMT pararelly and for specificity at 300,000 cells/ml when used serially (Table 15).

Table 15. Sensitivity and specificity values of SCC when used in combination with CMT, at different cutoff points taking as an example the case of crossbreds

No.	*Threshold /cutoff point cell/ml*	*Test method*	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
1	100,000	Parallel	98.67	42.09	22.66	99.46
		Serial	65.42	95.90	73.27	94.16
2	120,000	Parallel	96.70	50.38	25.09	98.88
		Serial	61.17	96.57	75.42	93.53
3	150,000	Parallel	96.04	59.99	29.21	98.80
		Serial	59.75	97.35	79.52	93.36
4	170,000	Parallel	96.04	63.30	31.03	98.94
		Serial	59.75	97.63	81.22	93.38
5	190,000	Parallel	95.37	66.28	32.72	98.81
		Serial	58.33	97.87	82.46	93.18
6	200,000	Parallel	94.71	66.62	32.78	98.65
		Serial	56.91	97.89	82.29	92.96
7	210,000	Parallel	94.05	68.27	33.76	98.52
		Serial	55.49	98.03	82.88	92.76
8	230,000	Parallel	93.39	70.92	35.58	98.42
		Serial	54.06	98.25	84.12	92.56
9	250,000	Parallel	93.39	71.58	36.10	98.44
		Serial	54.06	98.25	84.12	92.56
10	300,000	Parallel	93.39	71.58	36.10	98.44
		Serial	54.06	98.30	84.53	92.56

The relationship of CMT score and SCC is presented in Table 16 and 17. The mean SCC increased as CMT score increased.

Table 16. Relationship of CMT and SCC for crossbreds

CMT score	*Somatic Cell Count (SCC)*				
	No. of observation	Mean	Std. Dev.	Min	Max
0	263	167,821	206,581	7,859	1,391,043
trace	20	1,160,836	2,085,751	23,577	8,731,349
1	15	1,273,708	1,186,840	534,412	5,438,428
2	8	2,327,349	1,604,015	667,762	5,234,094
3	30	7,654,383	7,587,013	314,360	$2.83 * 10^7$

Table 17. Relationship of CMT and SCC for local zebu breds

CMT score	*Somatic Cell Count (SCC)*				
	No. of observation	Mean	Std. Dev.	Min	Max
0	671	125,217	175,925	0	3,033,224
trace	14	659,233	330,583	282,941	1,188,390
1	10	3,440,384	5,097,252	47,154	$1.23*10^7$
2	9	1,070,037	624,086	316,894	2,071,194
3	20	7,522,798	$1.05* 10^7$	339,529	$3.14* 10^7$

4.3.2. Factors affecting SCC

Six risk factors assumed to affect SCC were considered in this study. Factors included were infection status of the quarter, breed, stage of lactation, parity, quarter location and presence of tick and/or lesion on udder or teat. These factors were included in the model simultaneously and analyzed using two way ANOVA in Stata 7 software (Annex 6). The model was significant ($p < 0.05$). Statistically significance difference ($p < 0.05$) in linear score

(LS) i.e. the log ten transformed SCC was observed for infection status of the quarter, breed and stage of lactation. The remaining factors parity, quarter location, presence of tick and/or lesion on udder or teat were not significant ($p > 0.05$).

For significant factors having three levels, stage of lactation and infection status of the quarter, with t-test significant difference in linear score somatic cell count was observed between late stage of lactation (describe as coded 3 in statistical analysis part) and middle (coded 2) and early (coded 1) stage of lactation ($p < 0.05$). However, there was no difference between early and middle stage of lactation. High somatic cell count (linear sore of 5.27) occurred in late lactation compared to early and middle lactation having linear somatic cell sore of 4.96 and 4.88, respectively.

Similarly, significant difference was observed between uninfected quarter and infected quarter with minor or major pathogens ($p < 0.05$). Quarters harboring major pathogens showed high SCC (linear score of 5.77) compared to quarters infected with minor pathogens (linear score of 5.65) and uninfected quarter (linear score of 5.16). The difference between major and minor pathogens was not statistically significant ($p > 0.05$).

Crossbreds had high SCC (linear score of 5.28) when compared with local zebu breeds (linear score of 4.89) and the difference was statistically significant ($p < 0.05$).

In uninfected quarter (‘healthy’ udder), the effect of the above mentioned factors except infection status on SCC was tested (Annex 6). Still breed and stage of lactation was found to be significant ($p < 0.05$) where SCC in crossbreds and in late lactation stage was high.

5. DISCUSSION

5.1. Prevalence and Associated Risk Factors

A total of 351 cows, 195 lactating local zebu and 156 Holstein x Local Zebu crossbreds from private smallholder farms were investigated cross-sectionally.

Clinical prevalence at cow level was 3.9% in crossbreds and none in local zebu breeds. The clinical prevalence in crossbreds in this study was comparable with that of Bishi (1998) who reported 5.3% prevalence in Addis Ababa, Ethiopia. However, the present finding was lower than that reported by Workineh *et al.* (2002) (25.1%) in Addis Ababa, Ethiopia. Mastitis is a complex disease and the difference in results could be due to difference in management system between the farms. Subclinical mastitis was high in both breeds compared to clinical mastitis. The prevalence of subclinical mastitis in crossbreds at cow level based on CMT in the present study (34.4%) was similar to the finding of Bishi (1998) who reported 34.30%. However, Shirmeka (1996) reported a higher prevalence (40%) in the same study area compared to the present finding. Subclinical mastitis in local zebu breeds in the present finding (17.9%) was comparable to that of Gulima (1991) who found 14.8%, however, lower than that reported by Temesgen (1999) (25%) in Mekele, Tigray, Ethiopia.

In this study as well as in other similar studies, overwhelming cases of mastitis were subclinical compared to clinical mastitis in both breeds (Kassa *et al*., 1997; Hussein, 1999; Workineh *et al.,* 2002; Kerro and Tareke, 2003). In Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Hussein *et al.,* 1997) while the high economic loss could come from subclinical mastitis. Farmers in the study area usually complain about the decrease in milk yield irrespective of adequate feed provision and deworming practice (Elias, 2004 personal communication). According to Radostitis *et al.* (1994a), an infected quarter showed 30% and a cow 15% reduction in milk yield. Usually Ethiopian farmers specially smallholders are not well informed about the invisible loss from subclinical mastitis (Hussein, 1999) since dairying is

mostly a sideline business in these farmers. This was also true in the study area that none of the farmers screened their cows for subclinical mastitis except seeking veterinarian's assistance at times of clinical cases. To maximize milk production in the region, bureau of agriculture of the region should introduce systems that increase awareness on subclinical mastitis to farmers.

Among the risk factors considered to have effect on the occurrence of mastitis breed was found to be statistically significant ($p < 0.05$). The odds of occurrence of mastitis was three times more likely in crossbreds compared to local zebu. Increases in milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis (Schutz, 1994). Therefore, the lower prevalence in local zebu breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and McDaniel, 1985). In addition to physical barrier, difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001). In the present study, parity number 3 to 5 and late lactation stage were also found to increase occurrence of mastitis significantly ($p < 0.05$). According to Erskine (2001) primiparous cows have more effective defense mechanism than multiparous cows. The prevalence of subclinical infection increases as the stage of lactation progresses. These infections are generally the result of contagious mastitis and caused by an inability of mastitis control rather than a physiologic effect (Erskine, 2001). A significant number of isolates in this study were contagious pathogens.

5.2. Bacterial Isolation and Antibiotic Susceptibility Test

5.2.2. Bacterial isolation

Out of 1404 quarters examined, 54 (3.8%) were blocked indicating mastitis was a problem. In this Study, CNS were the predominant pathogens involved constituting 49.63% of all isolates. The high level isolation of CNS (49.63%) in this study closely agrees with the findings of Bishi (1998) and Hussein (1999) in Ethiopia who reported 54% and 42%, respectively. In Poland in a survey of mastitis in dairy herds of small-type farms in the Lublin region, CNS was isolated at a higher rate (36.6%) compared to other pathogens (Krukowski *et al.,* 2000). Comparable findings with the present study was also reported in Bolivia in mastitis survey by Edwards *et al*. (1987) where CNS was the predominant pathogen (55%) involved compared to others (Corynebacterium species 18%, *S. aureus* 12%, *Str. dysgalactiae* 6%, *Str. agalactiae* 4% and others 5%). Also in Denmark, out of 4645-quarter milk samples examined to determine the distribution of bacterial species in bovine mastitic milk, CNS were the second predominant isolate next to *S. aureus* (Aarestrup, 1995). Kerro and Tareke (2003) and Workineh *et al*. (2002), respectively, reported isolation of CNS at a rate of 2.5% and 30%, lower than the present study in a prevalence study in southern region and Addis Ababa, Ethiopia.

CNS are regarded as minor pathogens and normal inhabitants of bovine mammary gland and usually are mentioned in association with a slight increase in SCC (Rainard and Poutrel, 1988). However, recently CNS were isolated from bovine and other dairy animals mastitic milk samples (Ameh *et al*., 1993; Boscos *et al*., 1996; Almaw and Molla, 2000). Some studies indicated that CNS could be pathogenic and even cause more mastitis than *S. aureus*. Out of 834 cows with clinical mastitis in Ontario, Canada, 28.7% of the cases were due to Staphylococcus species (other than *S. aureus*) where as *S. aureus* was isolated only from 6.7% of the cases (Sargeant *et al*., 1998). In the present study, out of three clinical cases that yield bacterial growth, one case was due to CNS. According to Pyörölä (1991), over 30% of subclinical and nearly 20% of acute cases of mastitis were usually due to CNS.

The high isolation rate of CNS in this study could be associated with lowered resistance of the cow due to teat injury. Staphylococci typically colonize a broken skin and hence abrasion of the teat end increases the risk of staphylococcal colonization at the teat end and subsequent transfer into the udder (Pyörölä, 1991). In the present study, 164 out of 1350 (12.14%) functional quarters had tick and/or teat lesion. The overall quarter prevalence of mastitis in quarters with tick and/or teat lesion (4.3%) was high than in those without (3.03%) and out of the total isolates from quarters with tick and /or teat lesion, CNS took the higher proportion (40%). Actually, the effect of tick and/ lesion on the occurrence of mastitis in this study was not significant ($p < 0.05$) and this might be due to the low number of lesions (164/1350) observed in this study. Even if lesions observed during study the period were low, those inflicted earlier leading to establishment of infection might contribute to the high isolation rate of CNS in this study. CNS can be chronic and isolation from the same cow for a longer period is possible (Pyörölä, 1991). In a study conducted in Israel after experimental infection of quarters with CNS, it was possible to isolate CNS after months indicating CNS could result in chronic infection (Leitner, 2000). CNS were also credited for increasing the resistance of the colonized quarter to invasion by major pathogens and hence lowering the isolation rate of major pathogens relative to minor pathogens like CNS (Rainard and Poutrel, 1988). Generally, chronicity of CNS, presence of lesion and probably of the prophylactic role for major pathogens might explain the high rate isolation of CNS in this study.

The isolation rate of *S. aureus* (17.8%) in this study was the second next to CNS and was closely comparable with the findings of Bishi (1998) and Hussein (1999) who reported 9% and 10.69% prevalence in Addis Ababa, respectively. However, the present finding was lower than that of Workineh *et al*. (2002) and Kerro and Tareke (2003) where *S. aureus* accounted for 39.2% and 40.5% of the isolates, respectively, in their study at Addis Ababa and southern Ethiopia. The relatively high prevalence of *S. aureus* in this study could be associated with total absence of dry cow therapy and post milking teat dipping, the invariably hand milking practice, low culling rate of chronically infected cows (culling was usually due to feed shortage, aging and reproductive problem) and limited knowledge of farmers on segregation as a control option. The primary reservoir of contagious pathogens including *S. aureus* is infected quarter and the exposure of uninfected quarter is limited to the milking process (Fox and Gay, 1993).

In the present study out of 31 coagulase positive Staphylococci species 7 (22.58%) were *S. intermedius* and the remaining 24 (77.42%) were *S. aureus.* This finding was higher than that of Roberson *et al.* (1996) where out of 487 coagulase positive Staphylococci species from mastitic milk, 82.1% were *S. aureus*, 17.7% *S. hyicus* and 0.2% *S. intermedius.* Shibeshi (1998) reported lower than the present study where *S. intermedius* accounted for 1.65% of the isolates. Studies conducted so far on isolation of mastitis pathogens indicated that the rate of coagulase positive Staphylococcus other than *S. aureus* from cases of mastitis was very low if not totally absent (Miltenburg *et al.*, 1996; Wilson *et al*., 1997; Sergeant *et al*., 1998). Wilson *et al.* (1997) collected milk samples from 108,312 dairy cows from 1991 to 1995 in New York and Pennsylvania and found no coagulase positive Staphylococcus other than *S. aureus*. According to this report, over 75% of the intramamary infections were caused by S*tr. agalactiae*, other Streptococcus species, *S. aureus* and coagulase negative Staphylococcus species. However, some researchers said these pathogens are over looked and not well studied due to emphasis on *S. aureus*. According to Roberson *et al.* (1996) the prevalence and relevance of *S. aureus* intramamary infection have been well documented, however, importance of coagulase positive Staphylococci; S. *hyicus* and *S. intermedius* in udder health have not been firmly established. In routine mastitis microbiologic diagnostic procedures, all coagulase positive Staphylococci were usually taken to be *S. aureus* (Sears *et al.,* 1993). This procedure might under estimate the isolation rate of these two species.

Streptococci species were also among the dominant (16.3%) bacterial population as mastitis pathogens in Bahir Dar milk shed. *Str. agalactiae* (8.15%) and *Str. dysgalactiae* (6.67%) were the dominant species. *Str. uberis* (1.48%) was isolated at a lower rate. This finding was comparable with that of Kerro and Tareke (2003) who reported isolation rates of 13.1% *Str. agalactiae*, 5.6% *Str. dysgalactiae* and 5.1% *Str. uberis*. Bishi (1998) reported higher isolation rate (27%) for *Str. agalactiae* and lower (0.5%) for *Str. dysgalactiae* compared to the current finding. Bishi's finding on *Str. uberis* (1.9%) was closely similar to the present finding (1.48%). The explanation given for *S. aureus* could also be a factor for *Str. agalactiae* and *Str. dysgalactiae* relative high isolation rate since both of them are contagious pathogens.

In this study isolation rate of environmental pathogens for *Str. uberis* was low and none for enterobacteriacae specially coliforms. There is a common understanding that with increasing

herd size, manure disposal and sanitation problems also increase which will lead to build up of bacterial population (coliforms and environmental streptococci) in the cow's immediate environment (Saloniemi, 1991a). In this study, included were smallholder farmers who had average herd size of two lactating cows. The number of hours dairy cows kept indoor is also a factor that will increase the possibility of contact of teats with the environmental pathogens according to Saloniemi (1991a). Generally in smallholder management system, and was true in this study; that cows were allowed to graze for longer hours a day and supplemented with concentrate and hay when they return home late in the afternoon. This might minimize their stay indoor and hence minimal exposure rate to environmental pathogens. The low prevalence of clinical mastitis in this study could also be associated with this management system. In Israel dairy herds in one study, it was found out that most clinical mastitic cases were associated with coliform bacteria constituting 60.2% of the cases (Shpigel *et al*., 1998). In Netherlands, also environmental mastitis pathogens were found to be the most frequent isolates from clinical quarter cases (1103) in a random sample of dairy herds in southern Netherlands (Miltenburg *et al*., 1996).

5.2.3. Antibiotic susceptibility test

The purpose of this study was to identify effective antibiotics and to provide this information to veterinarians working in the study area. For a wider coverage, however, one protocol cannot be applied to all situations and more data on long-term antibiotic use must be collected before formulating any recommendations.

When comparing the overall efficacy of antimicrobials on all isolates, sulfisoxazole, erythromycin and clindamycin were the most effective antibiotic where 95% to 96.3% of the total isolates were found to be susceptible. Actually, these drugs were the least frequently used in the study area in the treatment of animals. According to Jaimes (2002), the development of antibiotic resistance nearly always has followed the therapeutic use of antimicrobial agents. The least effective drug was streptomycin where only 60.50% of the total bacterial population was found to be susceptible. Oxacillin also showed relatively weak

efficacy where 75.41% of the total isolates were susceptible. In this study, streptomycin showed very poor efficacy in almost all isolates.

Despite isolates tested in this study were all Gram positive bacteria and streptomycin is effective on Gram negative and on some Gram-positive bacteria (Carter and Chengappa, 1991), the poor efficacy of streptomycin in this study could be associated with the frequency of usage in the study region. Streptomycin was one of the most frequently used antibiotic to treat mastitis cases and other infectious diseases. The retrospective data collected from government veterinary clinic in the study area from 1999 - 2004 (5 years period), indicated that out of 182 mastitis cases brought to the clinic, 127 (69.78%) cases were treated with penicillin-streptomycin combination. Penicillin-streptomycin suspension is composed of procaine penicillin 200mg and dihydrostreptomycin sulphate 250mg. Rapid development of resistance occurs for streptomycin, rifampicin, erythromycin, oleandomycin, trimethoprim and novobiocin. With chloramphenicol and tetracycline, resistance develops more slowly as with penicillin (Pyörälä and Myllys, 1991). Equally, like streptomycin, penicillin was among the frequently used antibiotic in the treatment of cattle in the study area. However, due to inconveniences of availability penicillin was not included in this study. In the future research, it should be considered. For all cases of mastitis, (where in the study area therapeutic data collected) treatment was given based on presumptive diagnosis. For this reason, broad-spectrum antibiotics like penicillin-streptomycin combinations were used. However, antimicrobial susceptibility tests have been performed using individual agents. Susceptibility results of individual agents might not predict susceptibility to the combination of these compounds (Owens *et al*., 1997). Owens *et al.* (1997) found that penicillin and novobiocin combination was more effective on all isolates compared to individual results. The use of discs containing penicillin-streptomycin combinations in antimicrobial susceptibility tests in the present study area will be beneficial.

It is always worthy to discuss drug resistance with frequency of isolates as high proportion might be due to selection pressure. Pyörälä and Myllys (1991) stated that in areas where there is a tradition of using specific drugs there will be selection of drug resistance strains and during isolation the proportion of these isolates might be high. The dominant pathogens in this study were CNS, *S. aureus, Str. agalactiae* and *Str. dysgalactiae* in that order. CNS

isolates were susceptible to sulfisoxazole (95.45%), tetracycline (90.9%), erythromycin (95.45%), clindamycin (90.9%), and streptomycin (81.81%) but relatively resistant to chloramphenicol and oxacillin where isolates were 68.18% and 68.18% susceptible, respectively. In agreement with the present finding, Hussein (1999) reported erythromycin and sulfamethaxazole to be effective drugs on CNS. Bishi (1998) reported comparable results with the present finding in erythromycin and chloramphenicol but his result on tetracycline and streptomycin was different.

In this study, *S. aureus* isolates were most susceptible to sulfisoxazole (100%), chloramphenicol (100%), clindamycin (95.83%) and erythromycin (95.83%). Oxacillin and tetracycline were also effective on *S. aureus* and showed 91.66% and 89.28% susceptibility percentage, respectively. *S. aureus* showed resistance to streptomycin where only 29.16% of the total isolates were susceptible. Bishi (1998) obtained comparable results where erythromycin and oxacillin were 100% effective on *S. aureus* where as streptomycin was less effective where only 18% of the isolates found to be susceptible. Tetracycline and chloramphenicol were effective on *S. aureus* in the present study but not in Bishi (1998) where tetracycline and chloramphenicol were effective only on 12% and 18% of the isolates. The efficacy of erythromycin on coagulase positive Staphylococci according to Bezek (1998) was lower (73%) than the present finding (100%) on *S. aureus.* Owens *et al.* (1997) found *S. aureus* to be 100% sensitive to erythromycin, tetracycline, penicillin and cloxacillin that closely agreed to the present finding. The variability in susceptibility result could partly arise on how frequent a drug was in use in the study area. For example, according to Pyörälä and Myllys (1991) resistance of *S. aureus* strains isolated from mastitis infections to penicillin is less in the Nordic countries than in many other countries. In most countries, the Streptococci are susceptible to penicillin. There are, however, reports in the United States of considerable resistance to penicillin and other drugs among strains of mastitis Streptococci. This is a new phenomenon connected with frequent use of particular antibacterials (Pyörälä and Myllys, 1991).

From Streptococcus species *Str. agalactiae* and *Str. dysgalaciate* showed highest resistance to streptomycin (100% resistance). However, Hussein (1999) reported streptomycin to be effective on isolates of Streptococcus species. *Str. dysgalaciate* were also only 44.44%

sensitive to oxacillin. These two species were the frequently isolated pathogen among Streptococcus species and were susceptible to sulfisoxazole, tetracycline, erythromycin, chloramphenicol and clindamycin. According to Owens *et al.* (1997), *Str. dysgalaciate* were susceptible with degrees of 80%, 100% and 100% to cloxacillin, erythromycin and tetracycline, respectively. The higher isolation rate of some pathogens compared to others observed in this study, therefore, might not be due to selection pressure rather were as a result of other factors discussed in the preceding section.

5.3. Somatic Cell Count (SCC)

5.3.1. Factors affecting somatic cell count

The ability to correctly interpret SCC depends up on an understanding of the factors affecting it. Several studies were reviewed by Harmon (1994) and (Dohoo and Meek, 1982) and indicated the existence of variation in findings. Even these reviewers differ in their conclusion. According to Harmon (1994) except diurnal variation, few factors other than infection status have significant effect on milk SCC. According to him stage of lactation, age, season and various stressors have minimal effect if the quarter is uninfected, therefore, according to Harmon (1994) SCC could be used to determine intramamary infection. Dohoo and Meek (1982) reviewed the effects of infection status, age, stage of lactation, season, stress and diurnal variation. They concluded that somatic cell counts are general indicators of udder health and merely a reflection of udder damage due to variety of possible causes and as such indicate whether or not a pathogen is present.

To the author knowledge, no study has been carried out on factors affecting SCC under the existing environment, management and breeds in Ethiopia except the present attempt. In this study, the effect of breed, stage of lactation, parity, infection status of the quarter, quarter location and presence of tick and or lesion were evaluated. Out of these factors considered breed, stage of lactation and infection status were found to affect SCC significantly. Elevation

of SCC during infection was generally agreed up on and there are several reports supporting this (Harmon, 1994; Audist *et al.* 1995; Schepers *et al.,* 1997; Allore *et al*., 1998). However, reports vary on the significant effect of stage lactation. In the present study, significant difference was observed between late and middle and early stages of lactation, however, the difference between early and middle stages was not significant. Audist *et al.* (1995) reported elevated SCC during late lactation in agreement with the present finding. According to Audist *et al.* (1995), the increase in late lactation was probably a function of decreasing milk volume in combination of degeneration of the gland.

The important finding in this study was Holstein x Zebu had high SCC compared to local zebu and this has proved the existence of breed effect on SCC. According to Saloniemi (1991b), the breed of the cow influences SCC significantly. Finish Ayrshire have markedly lower SCC than Friesian cows. The low SCC observed in indigenous zebu compared to crossbreds in this study might be due to the genetic association between milk yield, SCC and resistance to mastitis. According to Schutz (1994), increases in milk yield from genetic selection may be accompanied by correlated increases in genetic susceptibility to clinical mastitis and somatic cell counts. Therefore it looks logical that local zebu known for their resistance to mastitis and low milk yield to have low SCC compared to crossbreds which are high milk yielding and susceptible to mastitis. However, this should be further evaluated in a properly designed study where the effect of factors other than breed are controlled.

5.3.2. Somatic Cell Count (SCC) threshold level

So far, culture is taken to be the best diagnostic method for mastitis. However, it is costly and labor intensive to use in routine diagnostic procedures. California Mastitis Test has been in use as a cow side test since many years back for screening subclinical mastitis but it is subjective and the result can vary from technician to technician (Schalm *et al.*, 1971). Somatic cell count is under consideration as an alternative and is in use in many countries. The environment, management and breed may affect the physiology of the cow, which in turn affects somatic cell count. Hence, variation may exist from country to country in setting SCC

thresholds. To the author knowledge to use SCC either in herd monitoring or in individual cow mastitis diagnosis, threshold levels have not been determined in Ethiopia.

Since breed was found to affect SCC, threshold level was evaluated for each breed independently. Ten cutoff values were considered taking mean SCC value of uninfected quarter as a reference taking values above and below. The selection of the threshold value or critical value depends on the element of the test that is most important to optimize. For this reason, generalized recommendation for threshold SCC will be difficult (Kirk *et al*., 1996; Erskine, 2001). For example, the use of SCC data to select cows for dry period therapy or to identify cows for culture would favor a high sensitivity with minimal false negatives (Erskine, 2001).

In this study, high sensitivity was observed at 80,000 cell/ml (sensitivity of 88.23% and specificity of 45.82%) for local breeds and at100, 000 cell/ml (sensitivity of 95.80% and specificity of 45.82%) for crossbreeds. At these threshold levels specificity was low and this goes with the theory that the relation between sensitivity and specificity changes with an inverse fashion (Kirk *et al.,* 1996).

Although comparison of the present study with Western countries could be difficult due to environmental, breed as well as methodology (microscopic method) differences, a slightly higher thresholds were reported. At 100,000 cell/ml cutoff, sensitivity and specificity with values of 83.2% and 80.5%, respectively, were reported by Schepers *et al*. (1997) in Holstein breeds. At the same threshold in the current study sensitivity and specificity were 95.80% and 45.52% in crossbreeds, respectively, which was different from Schepers *et al*. (1997). Hicks *et* a*l*. (1994) at 200000cells/ml threshold found 79% sensitivity and 72% specificity in Holstein cows in USA. This was closely comparable with the present finding in cross breeds at 200000cells/ml threshold where sensitivity and specificity were 83.33% and 72.04%, respectively. Also Schepers *et al*. (1997) at 200000 cell/ml threshold, reported sensitivity and specificity of 74.5% and 89.6%, respectively, a closely similar result with the present finding.

There is a controversy among researchers on the use of culture as a gold standard. Sears *et al*. (1993) after experimental infection with *S. aureus,* single milk culture showed only 74.5% sensitivity indicating intermittent shading. Culture was not only criticized for missing

intermittently shadding pathogens, but also the isolation may be the result of teat streak canal colonization or of overt contamination in the sampling process (Sears *et al.,* 1993, Erskine, 2001). However, Hicks *et al*.(1994) taking at least two of the three consecutive cultured samples yield similar result (pathogens) to be a gold standard, found 93% and 99% sensitivity and specificity, respectively, for culture of single milk sample. This finding indicated that single milk sample culture, the method used in the present study was still the best method to identify cows with intramamary infection compared to SCC and ELISA where these tests showed 79% and 72% and 69% and 61% sensitivity and specificity, respectively, in Hicks *et al*. (1994) study.

The sensitivity and specificity of SCC threshold value in the present study could have been improved if the gold standard for culture was isolation of the same organism from two samples from three consecutive samples, which will improve the quality of culture. Future research should take in to account use of three times sampling and isolation of the same organism from at least two samples to be the gold standard.

6 CONCLUSIONS AND RECOMMENDATIONS

This study attempted to quantify mastitis in Bahir Dar milk shed, isolate and determine antibiotic susceptibility profiles of pathogens involved and attempt to evaluate some cutoff points of SCC in identifying intramamary infection.

Subclinical mastitis was more important when compared to clinical mastitis. Crossbred cows were affected more than local zebu. The current breeding programme in Ethiopia should also emphasize on the importance of mastitis since in the years to come a significant percentage of dairy cattle population will be improved breeds.

The pathogens found involved were coagulase positive staphylococci (CNS), *S. aureus, Str. agalactiae, Str. dysgalactiae, Str. uberis,* Micrococcus species, *C.bovis, A. pyogens, B. cerus,* and *S. intermedius.* Among these, the most frequent isolates were CNS (49.63% of the total isolates), *S. aureus* (17.78%), *Str. agalactiae* (8.15%) and *Str. dysgalactiae* (6.67%). In this study, coliform bacteria were not isolated, which was inline with the low prevalence of clinical mastitis. The major isolates were contagious pathogens; therefore, careful milking practice such as use of single towel for each cow, disinfecting hands before milking and between milking and milking infected cows last should be followed.

All tested drugs showed resistance at least to one of the isolates, streptomycin was observed with poor efficacy. Sulfisoxazole and erythromycin could be the drug of choice for the study area. This study had limitation in including penicillin; therefore, future studies should include this drug as it was one of the most frequently used antibiotic in the treatment of cattle.

Anther important finding in this study was crossbreds had high SCC compared to locals, however, further study should be initiated to establish beyond doubt in a properly designed study where all factors other than breed will be controlled. In this study, infection status, breed and late lactation stage were found to increase SCC significantly.

A first attempt to establish threshold level to use SCC as a diagnostic tool in Ethiopia was made and different cutoff points were evaluated. However, to implement the use of SCC on a national scale further study is recommended with improved cultural methods.

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8. ANNEXES

Annex 1. Questionnaire format

Owners Name: _____Address_____

Date of sample Collection_____

1. Cow History:

Breed_____age___calvingdate____parity_____previous history of mastitis___

Tick infestation: present _____absent_____

Teat Lesion: present_____absent_____

Gross milk quality: watery____bloodtinged____clots/flakes___ normal___

Sample collected from: RR___RF____LF___LR_____

CMT score: RR___RF____LF___LR_____

2. Milking practice

Do you wash udder before milking? yes____ no____

Do you dry after washing? yes____ no____

Do you use the same cloth for both teats? yes____ no____

Do you practice milking mastitic cows last? yes____ no____

3. Housing

Floor concrete_____stone_____soil_____slopy_____leveled_____

Roof: metal sheet_____ grass_____

Wall: concrete_____mud_____other_____

Manure removal: daily_____weekly____monthly____other (specify)_____

4. Drug usage

Mention any drug you know used for treatment of any disease

_____

Name those used for mastitis treatment _____

Is there problem of cure after therapy? _____

You treat your animal by your self _____or take to the clinic_____

Annex 2. Interpretation of CMT findings

Source: Quinn *et al*. (1999)

Score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
T(Trace)	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickens with gel formation
3	Strong positive	Viscosity greatly increased .strong gel i.e. cohesive with a convex surface

Annex 3. Procedures for the identification of mastitis pathogens

Source**:** NMC (1990)

Differentiation of Mastitis Causing Staphylococcus and Micrococcus species

After inoculation on blood agar Gram positive cocci, catalase positives were taken to be Staphylococcus and Micrococcus species and were differentiated wwith (O – F test) . Micrococcus species are oxidative and Staphylococcus fermentative. Staphylococcus were further differentiated by coagulase test. Coagulase positive Staphylococcus species were further identied by VP. Stapthylococcus aureus were VP positive. VP negative Staphylococcus species were identified to be *S. intermedius*.

B. Differentiation of mastitis causing Streptococcal species

Species	CMP Test	Growth on MacConkey	Esculin hydrolysis	Other Confirmatory Tests
Str. agalactiae	+	-	-	
Str. uberis	+/-	-	+	Manitol +(A)
Str. dysgalactiae	-	-	-	Salicin +(A)
Str. faecalis	-	Pin point red colonies	+	
Str. pyogens	-	-	-	Salicin(-)
Str. pnuemoniae	-	-	+	Manitol(-)

C. Differentiating mastitis causing corynebacterium species

Test	A. pyogens	C.ulcerans	C.bovis
Haemolysis	+	+	-
Catalase	-	+	+
Growth in 9% NaCl	-	-	+

Annex 4. Procedures to conduct antibiotic susceptibility test

Source: Quinn *et al*. (1999).

Preparation of the inoculum

Inoculation of 6 to 7 distinct colony in to 5ml of saline was made first. Then the turbidity is compared with 0.5 MacFarland standard. This standard was prepared by adding 0.5 ml of 1 %(11.75g/litre) $Bacl_2.2H_20$to 99.5ml of 1 % (0.36N) H_2SO_4.

Inoculation to Mueller-Hinton agar

For slow growing bacteria, streptococci and corynebacterium species, 7% whole blood added Mueller-Hinton Agar was used.

A sterile cotton swab on a wooden applicator stick was used to transfer the diluted bacterial suspension to a plate; excess fluid was squeezed out by rotating the swab against the sides of the tube. The plate was seeded uniformly by rubbing the swab against the entire agar surface in three different planes.

Disc application

Within 15 minutes (time used to dry the inoculum) after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates by hand using a sterile forceps. All discs gently pressed down on to the agar with forceps to ensure complete contact with the agar surface. The discs were no closer than 1.5 cm to the edge of the plate and they were rest 3 cm apart from each other.

Incubation

The plates were incubated inverted aerobically for 24 hours at 37^0C

Interpretation

Inhibition zone was measured in millimeters using a transparent ruler on the under surface of the Petri dish. For measuring purpose, the end was taken as complete

inhibition of growth as determined by naked eye. The result was interpreted according to the Table presented below taken from Quinn *et al*. (1999).

Zone size interpretive chart for antimicrobials

Inhibition Zone Diameter (mm)

Antimicrobial agent	Disc potency	Resistance	Intermediate	susceptible
Streptomycin S10	10µg	≥11	12-14	≤15
Tetracycline TE30	30µg	≥14	15-18	≤19
Erythromycin E15	15µg	≥13	14-17	≤18
Penicillin G10 for staphylococci	10U	≥20	21-28	≤29
Penicillin G10for othermicroorganisms	10U	≥11	12-21	≤22
Gentamycin CN 10	10µg	≥12	13-14	≤15
Chloamphinicol C30	30µg	≥12	13-17	≤18
Polymyxin B PB30	300U	≥8	9-11	≤12
Novobiocin*	30µg	≥17	18-21	≤22
cloxacillin	30µg	≥		≤
Kenamycin K30	30µg	≥13	14-17	≤18
Oxacillin	1µg	≥10	11-12	≤13

*Not applicable to media that contain blood

Annex 5. Stata out put for risk factors affecting prevalence of mastitis

A) Univariate logistic analsis

Syntax:. logistic cmtsco breed

Logit estimates Number of obs = 1010

LR chi2(1) = 27.15

Prob > chi2 = 0.0000

Log likelihood = -305.88101 Pseudo R2 = 0.0425

cmtsco	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
breed	3.140831	.6814276	5.28	0.000	2.052909	4.805287

Syntax: logistic cmtsco stlac

Logit estimates Number of obs = 1010

LR chi2(1) = 20.73

Prob > chi2 = 0.0000

Log likelihood = -309.09261 Pseudo R2 = 0.0324

cmtsco	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
stlac	1.815218	.2427536	4.46	0.000	1.396676	2.359186

Syntax: logistic cmtsco parity

Logit estimates Number of obs = 1010

LR chi2(1) = 3.68

Prob > chi2 = 0.0552

Log likelihood = -317.61727 Pseudo R2 = 0.0058

```
------------------------------------------------------------------------------
      cmtsco | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      parity |    1.39136   .2359715     1.95   0.051     .9978784    1.939998
------------------------------------------------------------------------------
```

Syntax: logistic cmtsco ticklesion

```
Logit estimates                           Number of obs   =       1010
                                          LR chi2(1)      =       2.18
                                          Prob > chi2     =     0.1394
Log likelihood = -318.36345               Pseudo R2       =     0.0034

------------------------------------------------------------------------------
      cmtsco | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  ticklesion |   1.674234   .5562135     1.55   0.121     .8730319    3.210717
------------------------------------------------------------------------------
```

Syntax: logistic cmtsco quarterloc

```
Logit estimates                           Number of obs   =       1010
                                          LR chi2(1)      =       0.02
                                          Prob > chi2     =     0.8987
Log likelihood = -319.44774               Pseudo R2       =     0.0000

------------------------------------------------------------------------------
      cmtsco | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  quarterloc |   1.027564   .2194756     0.13   0.899     .6760865    1.561765
------------------------------------------------------------------------------
```

B) Multiple logistic regression

Syntax: logistic cmtsco breed stlac parity ticklesion

```
Logit estimates                           Number of obs   =       1010
                                          LR chi2(4)      =      53.35
                                          Prob > chi2     =     0.0000
Log likelihood = -292.77862               Pseudo R2       =     0.0835

------------------------------------------------------------------------------
      cmtsco | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       breed |   3.091657   .6886433     5.07   0.000     1.997986    4.783987
       stlac |   1.928416   .2727565     4.64   0.000     1.461525    2.544458
      parity |   1.577942    .291352     2.47   0.013     1.098816    2.265987
  ticklesion |   .9903112   .3548713    -0.03   0.978     .4906246    1.998914
------------------------------------------------------------------------------
```

C) Chi square comparisons for variables having levels

Syntax: tabulate stage1 stage2,chi2

```
           |       stage 2
   stage 1 |         0         1 |     Total
-----------+----------------------+----------
         0 |       267        23 |       290
         1 |        24         0 |        24
-----------+----------------------+----------
     Total |       291        23 |       314

          Pearson chi2(1) =   2.0539   Pr = 0.152
```

.Syntax: tabulate stage1 stage3,chi2

```
           |       stage 3
```

stage 1	0	1	Total
0	220	31	251
1	11	13	24
Total	231	44	275

Pearson chi2(1) = 28.4996 Pr = 0.000

.Syntax: tabulate stage2 stage3,chi2

stage 2	stage 3 = 0	stage 3 = 1	Total
0	217	44	261
1	14	0	14
Total	231	44	275

Pearson chi2(1) = 2.8097 Pr = 0.094

. Syntax: tabulate parity1 parity2,chi2

parity 1	parity 2 = 0	parity 2 = 1	Total
0	317	48	365
1	11	6	17
Total	328	54	382

Pearson chi2(1) = 6.5619 Pr = 0.010

.Syntax: tabulate parity1 parity3,chi2

```
          |       parity 3
 parity 1 |         0         1 |     Total
-----------+----------------------+----------
         0 |        49         0 |        49
         1 |         3         2 |         5
-----------+----------------------+----------
     Total |        52         2 |        54

          Pearson chi2(1) =  20.3538   Pr = 0.000
```

. Syntax: tabulate parity2 parity3,chi2

```
          |       parity 3
 parity 2 |         0         1 |     Total
-----------+----------------------+----------
         0 |        37         0 |        37
         1 |        15         2 |        17
-----------+----------------------+----------
     Total |        52         2 |        54

          Pearson chi2(1) =   4.5204   Pr = 0.033
```

.

Annex 6. Stata out put for risk factors affecting somatic cell count (SCC)

A) Syntax: anova ls ticklesion parity stlac breed culture quarterloc

Source	Partial SS	df	MS	F	Prob > F
Model	99.3631481	9	11.0403498	16.30	0.0000
ticklesion	.128048068	1	.128048068	0.19	0.6638
parity	3.07499595	2	1.53749798	2.27	0.1038
stlac	24.9736966	2	12.4868483	18.44	0.0000
breed	16.6954988	1	16.6954988	24.65	0.0000
culture	37.0331093	2	18.5165547	27.34	0.0000
quarterloc	1.76278226	1	1.76278226	2.60	0.1070
Residual	677.31287	1000	.67731287		
Total	776.676018	1009	.769748283		

B) SCC For healthy quarters to see whether there has been a carry over effect from infection from other factors

Syntax: anova ls ticklesion parity stlac breed culture quarterloc

Number of obs = 1010 R-squared = 0.1279
Root MSE = .82299 Adj R-squared = 0.1201

Source	Partial SS	df	MS	F	Prob > F
Model	99.3631481	9	11.0403498	16.30	0.0000
ticklesion	.128048068	1	.128048068	0.19	0.6638
parity	3.07499595	2	1.53749798	2.27	0.1038
stlac	24.9736966	2	12.4868483	18.44	0.0000

```
    breed |  16.6954988     1   16.6954988       24.65     0.0000
  culture |  37.0331093     2   18.5165547       27.34     0.0000
quarterloc |  1.76278226     1   1.76278226       2.60     0.1070
           |
 Residual |   677.31287  1000   .67731287
-----------+----------------------------------------------------
    Total |  776.676018  1009  .769748283
```

9. CURRICULUM VITAE

Name : Gizat Almaw

Sex : Male

Date of birth: September 15, 1974

Place of birth: Dembecha, Gojjam, Ethiopia

Nationality : Ethiopian

Marital status: Single

Profession : Veterinarian

Education

1978-1991 Arbegnoch elementary school, Yecherequa

1990-1991 Dembecha Junior Secondary and Senior Secondary School

1991-1994 Dembecha secondary high school, Dembecha

1994-1999 Faculty of Veterinary Medicine, Addis Ababa University

Work Experience

2000-2002 District Veterinary Practitioner, Bureau of Agriculture of the Amhara National Regional State

Publication

Almaw, G. and Molla, B. (2000): Prevalence and etioilogy of mastitis in camels (Camelus dromedarius) in eastern Ethiopia. J. Camel Pract. Res. 7(1), 97-100.